II. UPDATE OF INFORMATION RE PATENT APPLICATION

Subsequent to the filing of the Response to Office Action mailed November 9, 2004 and the response thereto, parent application Ser. No. 09/397,110 has become U.S. Patent 2,682,497 issued November 30, 2004.

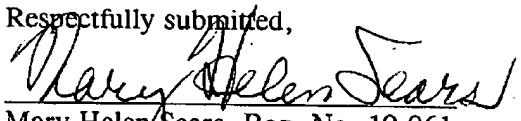
There are accordingly supplied revised pages 4, 5, 6, 7, 8 and 11 each of which previously referred to the application number but not its patent number and filing date. On new pages 5 and 7, at the top of each, we have underlined as new to that page words carried over from original pages 4 and 6 , respectively, as a result of computer shifting of the last line on the original page.

III. Further Remarks

The claims erroneously numbered previously as 9-14 herein are again presented as 10-15 respectively. Non-elected claim 9, which was inadvertently overlooked in responding to the Office Action of July 27, 2004 has been cancelled.

The further responses to substantive rejections remain as set forth in the original response to that action.

Respectfully submitted,



Mary Helen Sears, Reg. No. 19,961
Attorney for Applicants
The M.H. Sears Law Firm, Chartered
910 Seventeenth Street, N.W.
Washington, D.C. 20006
Telephone: 202-463-3892
Telecopy: 202-463-4852

CORRECTED APPLICATION PAGES

assay in urine, *Chest* 2001, vol. *119,* 243-9; Yu V.L., Kellog, J.A, Plouffe, J.F. *et al,* Evaluation of the Binax Urinary, Gram stain and sputum culture for *Streptococcus pneumoniae* in patients with community-acquired pneumonia, 38th Annual Meeting of the Infectious Disease Society of America, New Orleans, LA, Abstract #262 (2001).

The NOW® bioassay is described and claimed in co-pending, commonly assigned U.S. Patent Application Serial No. 09/397,110 filed September 16, 1999, which is incorporated herein by reference, and is now U.S. patent 6,824,997 issued November 30, 2004, and also its parent application Serial No. 09/156,486 filed September 18, 1998 and now abandoned.

A study of pneumonia conducted in China found that children with nasopharyngeal carriage of *Streptococcus pneumoniae* had high rates of positive urine results in the NOW® test even when they had no pneumonic disease and that the test results accordingly did not fit the sensitivity and specificity profile established with adult subjects. A study in Gambia found that 87% of well children tested were nasopharyngeal carriers of *Streptococcus pneumoniae* and that 55% of these, or about 47% of this population, gave false positive results in the Binax NOW® test. See Adegbola, R.A., Obaro, S.K., Biney, E. and Greenwood, B.M., Evaluation of Binax NOW ® *Streptococcus pneumoniae* urinary antigen test in children in a community with a high carriage rate of pneumococcus, Pediatr. Infect. Dis. J. 2001, July; 20 (7) 718-719. See also Dowell, S.F., Garman, R.L., Liu, G., Levine, O.S. and Yang, Y.H., Evaluation of Binax NOW as assay for the detection of pneumococcal antigen in urine samples performed among pediatric patients, Clin Infect Dis. J. 2001, vol. *32,* 824-825 (2001). A similar study conducted among 210 children in Quito, Ecuador, confirmed that urine from children with nasopharyngeal carriage of *Streptococcus pneumoniae* gives a high proportion of false positive

results in the Binax NOW® test. See Hamer, D., Egas, J., Estrella, B., MacLeod MacLood, W. *et al*, 2002, An assessment of the Binax NOW *Streptococcus Pneumoniae* urinary test in children with Nasopharyngeal pneumococcal colonization, (Publication in press)

An article reviewing published studies performed on Scandinavian and Israeli children confirms that young children in these areas have a high rate of nasopharyngeal colonization, not only of *Streptococcus pnuemoniae* but also of the bacteria that are known to cause disease states that resemble pneumococcal pneumonia, including especially non-typable *Haemophilus influenzae* and *Moraxella catarrhalis* which, with *Strepococcus pneumoniae,* are the most common causes of otitis media. Among other agents that tend to colonize the nasopharynx and are causatives of both pneumonic illness clinically very similar to pneumococcal pneumonia and otitis media are *Staphylococcus aureus*, a number of other bacteria and some viruses. See Harper, M.B., Nasopharyngeal colonization with pathogens causing otitis media; how does this information help us? Pediatr Infec. Dis. J. vol. *18,* 1120-1124 (1999)

Copending, commonly assigned U.S. application Serial No. 09/518,165 filed March 1, 2002, describes and claims rapid immunochromatographic tests for detecting bacterial carbohydrate antigens in human bodily fluids, including urine.

The methodology for lessening and/or eliminating false positives in child carriers who are colonized nasopharyngeally as described herein is applicable to the modification of tests for antigens of other bacteria which tests are disclosed in copending, commonly assigned application Serial No. 09/518,165 as well as to the test for *Streptococcus pneumoniae* antigens described in copending commonly assigned application Serial No. 09/397,110, now U.S. Patent 6,824,997.

In general, the development of rapid, reliable, specific and sensitive assays for antigens of bacteria causative of common respiratory tract and ear infections in children--and especially pneumonia and otitis media because of their high incidence--is important to complement the strategies that the Centers for Disease Control in the United States and the World Health Organization globally have formulated for decelerating the pace of development by causative bacteria of strains resistant to antibiotic therapy.

BRIEF DESCRIPTION OF THE INVENTION

The present invention involves modifying of the Binax NOW® immunochromatographic ("ICT") antigen test for use with young children, especially in geographic areas where nasopharyngeal colonization of these children with *Streptococcus pneumoniae* is a significant clinical manifestation, to markedly diminish or eliminate false positive test results that have been obtained when testing the urine of non-diseased children who are nasopharyngeal carriers of *Streptococcus pneumoniae*. The invention encompasses making analogous modifications of other immunoassay tests for other antigens characteristic of *Streptococcus pneumoniae* and antigens characteristic of other bacteria that both (1) are causative of pneumonic disease and otitis media in young children and (2) tend to colonize the nasopharynx in uninfected children.

The test modifications rest upon the unexpected discovery that, in general, nasopharyngeal carriage of disease-causing bacteria results in lower concentrations in bodily fluids, including urine, of target bacterial antigens for the ICT tests described in earlier filed, copending patent applications U.S. Serial No. 09/397,110, now U.S. Patent 6,824,997 and

U.S. Serial No. 518,165, than the concentrations of the same antigens found in bodily fluids of children infected with pneumonic disease or otitis media.

The modified tests employ reduced concentrations of antibodies to the target bacterial antigens.

The objective of the modifications, which is to maintain high specificity for diseased patient samples and to improve sensitivity to those samples by screening out samples from healthy, but nasopharyngeally colonized, children which gave false positives in the standard NOW® test for *Streptococcus pneumoniae*.

DETAILED DESCRIPTION OF THE INVENTION

The NOW® bioassay for identifying the characteristic C-polysaccharide antigen of *Streptococcus pneumoniae* present in all serotypes of these bacteria, has been demonstrated to be highly satisfactory in enabling physicians to make rapid, accurate diagnoses of a variety of *Streptococcus pneumoniae* - caused disease states in adults by coordinating carefully observed clinical symptoms with the test results. This ICT test is described and claimed in commonly assigned, copending U.S. patent application Serial No. 09/397,110, now U.S. Patent 6,824,997. U.S. application Serial No. 09/518,165, also copending and commonly assigned, discloses how to construct and perform analogous ICT bioassays which target characteristic carbohydrate antigens of other bacteria, including but by no means limited to non-typable *Haemophilus influenzae, Moraxella catarrhalis,* and *Staphylococcus aureus*.

The modifications disclosed herein of the NOW® test disclosed and claimed in U.S. Patent 6,824,997 render the test as so modified highly useful in enabling physicians to make rapid, accurate diagnoses of pneumococcal pneumonia and/or otitis media caused by *Streptococcus pneumoniae* in children, which diagnoses are based on the modified test results combined with clinical observations of the individual patients. Analogous modifications of the tests covered in U.S. Serial No. 09/518,165 render those tests as so modified very useful in enabling physicians to make rapid, accurate diagnoses of pneumonic diseases and otitis media of other bacterial origin in children, by combining the modified test results with clinical observation of individual child patients. Similar modifications may be made to any bioassay for an antigen characteristic of bacteria that tend to colonize nasopharyngeally in children and are causatives of pneumonic disease and/or otitis media, in order to improve diagnostic reliability on the assay results by diminishing or eliminating false positive results in children due to nasopharyngeal colonization.

To put the specific modified tests described in the examples of this application in perspective, a brief summary of the bioassay format described in both of the prior copending applications is provided. Succinctly, antibodies to the target bacteria are obtained by conventionally injecting a laboratory animal with the bacteria and conventionally obtaining from the animal a blood sample containing antibodies to the injected bacteria after a suitable time interval. Meanwhile, there is obtained from a culture of the same bacteria by a purification process described in the copending applications, an essentially protein-free carbohydrate antigen characteristic of these bacteria. The thus-purified antigen is coupled to a chromatographic column and the antibodies from the animal are rendered antigen-specific by

In all of these examples, test strips were prepared as described in earlier filed, copending Serial No. Application 09/397,110, now U.S. Patent 6,824,997 using antibodies to *Streptococcus pneumoniae* that had been purified and rendered antigen-specific as described in that application. In all of these tests, the capture lines were striped on the test strip membranes by passing each of them under the delivery tip of a precision pump system at a rate of 0.5 ml. per 6 mm. of membrane.

EXAMPLE 1
Reducing Capture Line Concentration Only

In this example, the concentration of the capture line was reduced from the 1.25 mg./ml. normally used to each of the concentrations shown in the table. The optical density of the gold-purified antibody conjugate which is indicative of concentration, was maintained at 2.0 The test results appear in Table 1:

TABLE I

Antibody Concentration of Capture Line	Carriers Giving Negative Result	Samples POSITIVES % Positive
0.3 mg./ml.	1 of 7 = 14% samples	100%
0.5 mg./ml.	4 of 11 = 36% samples	100%
1.0 mg./ml.	1 of 11 = 9% samples	100%